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A BEHAVIORAL DEFENSE AGAINST DISEASE

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Front Cover: Averaged images of the faces (sick and healthy) used as stimuli in Studies 1 and 2. Images made by Audrey Henderson, MSc, St Andrews University.

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A Behavioral Defense Against Disease

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In loving memory of my mother

ABSTRACT

Animals, including humans, have evolved under the continuous selection pressure posed by pathogens. As a result, we have developed a set of physiological mechanisms to combat pathogens – the immune system. However, engaging the immune system in this battle can be costly, unnecessarily so in cases when pathogens can be avoided. Recent studies have focused on the behavioral defense against disease, which helps us to avoid pathogens before they enter the body by detecting and avoiding sources of contagion. A behavioral defense can also be argued to promote recovery if infected, through so-called sickness behavior. Both aspects are related to motivational states that help the organism reorganize its priorities to promote disease avoidance and recovery. In this thesis, we investigated by which perceptual cues, facial and/or olfactory, humans are able to dissociate between sick and healthy individuals as well as how these cues can affect social liking of other people. We also investigated whether sick individuals would exhibit sickness behavior that includes relevant shifts in chemosensory perception of food and social odors.

In **Study I**, we investigated whether facial expressions of emotion change during sickness. Twenty-two healthy volunteers were injected with either an endotoxin (lipopolysaccharide, LPS; 2ng/kg body weight) or saline. Facial photographs of the volunteers were taken 2 hours after the injections. At a later stage, 49 naïve participants were asked to rate the emotional expressions and the perceived health of both the sick and healthy faces. The results showed that sickness had a negative effect on facial expressions of emotion. Sick faces looked significantly more sad and disgusted, as well as less happy, compared to the healthy faces. Moreover, the emotional expressions mediated 59.1% of the treatment-dependent changes in ratings of the perceived health of the faces.

In **Study II**, using the sick and healthy facial photographs from Study I and body odors from the same volunteers, we investigated the effects of olfactory and facial (visual) disease cues on social liking. We also assessed whether individual traits such as perceived vulnerability to disease, disgust sensitivity, and health anxiety could influence participants' liking ratings. Seventy-seven participants were presented with sick and healthy facial photographs and body odors in a 2 x 2 factorial design. During the presentation of the stimuli, facial electromyographic activity was recorded as an objective measure of participants' own facial expressions of emotion. The results revealed a negative main effect of both facial and body odor sickness cues on liking ratings, indicating that sick individuals are liked less than their

healthy counterparts. No significant effect of sickness cues on facial electromyographic activity was found. Finally, we showed that participants who perceived themselves as more vulnerable to disease liked the presented volunteers less [than other participants did], regardless of health status.

In **Study III**, we sought to examine if, apart from experimentally induced inflammation, naturally occurring inflammation can result in an altered body odor. As in Study II, the effect of individual traits – perceived vulnerability to disease, disgust sensitivity, and health anxiety – on sickness perception was also assessed. Body odors were collected from 23 volunteers who were experiencing respiratory infections. Three weeks later, body odors from the same individuals were collected again, after they had recovered. In a later stage, 46 participants rated the body odors in terms of intensity, disgust, pleasantness, and health. Our results showed that the sick body odors, in line with hypotheses, smelled nominally more intense, more disgusting, and less healthy compared to the healthy body odors, though these results did not reach statistical significance. Moreover, there was no association between the individual traits and body odor perception.

In **Study IV**, we assessed the effect of sickness on odor and taste perception. Relevant to this study is the fact that sickness behavior entails both a loss of appetite and social withdrawal. Using an experimental disease model, 40 participants received LPS (between 0.51 and 0.80 ng/kg body weight) or saline injections. They were then presented with eight different odors (two food odors, three social odors, and three control odors) and four different basic tastes (plus a control) and asked to rate the intensity and pleasantness of these stimuli. In line with the hypothesis, participants perceived food odors as significantly less pleasant when they were sick. No significant effect of sickness on ratings of social odors, control odors, or tastes was found.

In conclusion, the present thesis reveals a number of perceptual sickness cues with the potential to trigger avoidance or limit approach behavior. These cues are available as early as a few hours after the induction of systemic inflammation. Once sick, an individual demonstrates less motivation to consume food, as indicated by a dampened perception of food odor pleasantness, likely in favor of allocating resources to recovery. Avoiding the sickness cues and engaging in the sickness behaviors identified in this thesis may be important in a behavioral defense against disease that helps us stay alive and healthy.

LIST OF SCIENTIFIC PAPERS

I. **Sarolidou, G**., Axelsson, J., Sundelin, T., Lasselin, J., Regenbogen, C., Sorjonen, K., Lundström, J. N., Lekander, M., Olsson, M. J. (2019). Emotional expressions of the sick face. *Brain, Behavior, and Immunity*, 80, 286-291. doi: 10.1016/j.bbi.2019.04.003

II. **Sarolidou, G**., Axelsson, J., Kimball, B. A., Sundelin, T., Regenbogen, C., Lundström, J. N., Lekander, M., Olsson M. J. (in press). People expressing olfactory and visual cues of disease are less liked. *Philosophical Transactions of the Royal Society B.*

III. **Sarolidou, G**., Tognetti, A., Lasselin, J., Regenbogen, C., Lundström, J. N., Kimball, B. A., Lekander, M., Axelsson, J., Olsson, M. J. Olfactory communication of sickness cues in respiratory infection. *Manuscript submitted for publication.*

IV. **Sarolidou, G**., Tognetti, A., Lasselin, J., Schedlowski, M., Ohla, K., Lekander, M., Olsson, M. J. Sickness behavior and chemosensory perception. *Manuscript in preparation*.

CONTENTS

LIST OF ABBREVIATIONS

DS-R	Disgust Scale Revised
EMG	Electromyography
fMRI	functional Magnetic Resonance Imaging
HAI	Health Anxiety Inventory
IL	Interleukin
LPS	Lipopolysaccharide
PRRs	Pattern recognition receptors
PVD	Perceived Vulnerability to Disease
TLR	Toll-like receptors
TNF-α	Tumor necrosis factor alpha
VAS	Visual Analogue Scale
VOC	Volatile organic compound

1 INTRODUCTION

I have no idea what's awaiting me, or what will happen when all this ends. For the moment I know this: There are sick people and they need curing.

Albert Camus, The plague

August 31, 1854, Soho District, London. The people of Broad Street were hit by the worst cholera outbreak that had ever occurred in the United Kingdom, accounting for 616 deaths. The London Medical Community believed that cholera is caused by particles in the air, *miasma* (from the Greek word "μίασμα", meaning pollution). However, the physician John Snow had a different opinion; cholera, in his opinion, is caused by an unidentified germ cell that contaminated food or water. And that was, indeed, the case. The cholera outbreak was caused by germ-contaminated water.

That was not the first time that humans had suffered an epidemic disease, or, even worse, a pandemic. The Black Death (Bubonic Plague) in Europe in the late Middle Ages was the worst plague outbreak, killing millions of the European human population (Lippi & Conti, 2002).

Cholera and Plague are just two examples of recent history's battle between parasites and humans. This battle, though, goes back thousands of thousands of years. Pathogens were present on the planet long before the first modern human appeared, and, more importantly are still almost impossible to fully avoid. A lost battle someone might think, but they could not be more wrong.

Pathogens have been a major cause of death among living organisms (Inhorn & Brown, 1990). However, humans' response to them has also been the target of selection pressures. These selection pressures in turn, have resulted in the gradual development of the sophisticated defenses collectively referred to as the *Immune System*. With a functional immune system, humans can confront many pathogens that enter their bodies.

The ability to detect pathogens before they even enter the body seems the ideal solution to overcome the threat of contamination. So, apart from the protection the immune system offers against pathogens, humans have evolved two different types of behavioral defenses against disease. These types of behavior include avoidance of pathogens before they enter the body, often referred to as the Behavioral Immune System (Schaller, 2006) and, once infected, changing behaviors in order to promote recovery, known as *sickness behavior* (Robert Dantzer, 2009).

The present thesis will investigate both types of behaviors that are involved in the defense against pathogens. The thesis will focus on whether humans are able to detect subtle cues of disease in others and whether these cues are of the nature that we could expect or observe avoidance reactions. Sickness behavior that takes place once we are infected will also be examined. In the following chapter I will briefly give an overview of the immune system. Next, I will describe the mechanisms that are employed when a disease threat is present, including disease avoidance behaviors. Finally, I will also explain what types of behavior are initiated when we get sick (sickness behavior).

2 THE IMMUNE SYSTEM

"That which is used – develops. That which is not used wastes away."

Hippocrates

As noted, infectious diseases have exerted a heavy selection pressure on human development resulting in a sophisticated immune system (Fumagalli et al., 2009; Schaller & Park, 2011) which is crucial for our survival. Involving various biological structures, such as tissues, proteins, cells, and organs, it is distributed over the whole body. Among its many challenges, it is expected to detect pathogens, differentiate between the body's own tissue and foreign tissue, and it continuously learns and develops.

2.1 INNATE AND ADAPTIVE IMMUNITY

Our immune system can be classified in two categories; innate immunity and adaptive immunity. One of the differences between innate and adaptive immunity is the time each type takes to react to pathogens. Innate immunity is non-specific and acts immediately when a pathogen enters the body whereas adaptive immunity needs time to develop but provides specific response against pathogens.

The innate branch of the immune system is thus the first step for combatting pathogens. Epithelia of the skin act as a first line of defense by providing mechanical and chemical barriers, and can together with other barriers block pathogens from entering the body. If, however, pathogens break the epithelial barriers and reach other tissues, cells of the innate immune system, such as macrophages and granulocytes, attack them. Epithelial barriers, phagocytes, dendritic cells and mast cells are all important parts of innate immunity. In order to respond to pathogens, cells of the innate immune system use pattern recognition receptors (PRRs). A certain type of PRRs, called toll-like receptors (TLR), respond to both bacteria and virus. Within this family of receptors dedicated to respond to microbial infection, TLR4 is central in recognizing lipopolysaccharide (LPS; “endotoxin”), which is a component of gram-negative bacteria (Beutler, 2009). This makes it possible to administer LPS to cause a sterile inflammatory activation to mimic a bacterial infection, thus providing the ground for an experimental sickness model (see Chapter 6). After phagocytosis (when an innate immune cell such as a macrophage has “engulfed” a foreign microbe), the cell presents a small fragment of the processed material to another group of white blood cells, the lymphocytes. This links over to the adaptive immune system, so that cells with specific receptors for specific microbes can be activated and start to multiply, a process called clonal expansion.

Unlike the innate immune system, the adaptive immune system does not respond to an antigen as fast, but is more efficient against pathogens after which immunization has taken place. The adaptive immune system plays a crucial role in defending the body from pathogens that could resist an innate immune response, and renders a targeted attack towards an antigen possible.

There are two types of adaptive immunity: humoral and cell-mediated. Humoral (i.e. related to body fluids) immunity is mediated by B lymphocytes that are responsible for producing antibodies. These are soluble proteins that can perform multiple functions of those required to neutralize pathogens. For example, coating of pathogens with antibody increases the effectiveness of innate immunity (Abbas, Lichtman, & Pillai, 2014).

Cell mediated immunity (immunity dependent on cell-to-cell contact rather than through antibodies) combats viruses but also other targets and is mediated by T lymphocytes. There are many T cells subclasses, such as Cytotoxic, Helper, Regulatory T-cells, each of which with specialized functions (Hoeppli, Wu, Cook, & Levings, 2015).

If an intruder like a virus enters a cell and cannot be reached by antibodies, so called natural killer cells (NK cells) can be used in addition to T-cells. The NK cells are fundamental in the innate branch of the immune system, but thus also have an important role in adaptive immunity.

Through adaptive immunity, immunological memory can develop, so that quick and specific recognition of a previously encountered antigen can occur and an immune response be launched when needed. An immune response can also be developed through vaccination. That way B-cells produce the relevant antibodies and the organism can consequently be protected from the corresponding infections (e.g. smallpox).

2.2 INFLAMMATION

Inflammation can be either local or systemic. Local inflammation refers to the tissue reaction during which host defense cells are driven to infection sites. There are five classic main signs of local inflammation; redness, warmth, pain, swelling, and disturbance of function. These are caused by a local increase in blood flow and triggering of nerve endings in response to tissue damage. On the other hand, systemic inflammation is an inflammatory process that is characterized by the presence of pro-inflammatory cytokines in the plasma (Zotova, Chereshnev, & Gusev, 2016).

Systemic inflammation is caused by the release of pro-inflammatory cytokines. Cytokines are small proteins that are central players in an inflammatory response. Cytokines are mainly generated by immune cells and can have either pro- or anti-inflammatory effects. Specifically, cytokines can be classified in pro-inflammatory, such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor (TNF), that promote cellular immune response, and anti-inflammatory, such as interleukin-10 (IL-10), which down-regulate the pro-inflammatory cytokines (Cavaillon, 2001). Perhaps not surprisingly, the general dichotomization in pro- or anti-inflammatory cytokines is a simplification. For example, IL-6 has both pro- and anti-inflammatory functions (Del Giudice & Gangestad, 2018), even though it is most often reported as part of the pro-inflammatory class.

Even though the immune activation may save our lives, it comes at a cost. Mounting an immune response demands a great amount of energy from the body, resources that would otherwise be used for other body functions (Brown, 2003; Klein & Nelson, 1999), and, thus, during sickness we might feel dysfunctional and vulnerable.

3 A BEHAVIORAL DEFENSE AGAINST DISEASE

"Man only selects for his own good..."

Darwin, Origin of Species

When humans and animals mount an immune response, that response comes at a certain metabolic cost for the body. Immune responses are also *reactive*, meaning that they are initiated only after a pathogen enters the body. It has been suggested, as noted, that humans have also a behavioral defense that acts against disease and can therefore improve immunity. This behavioral defense is comprised of *proactive* mechanisms that are activated before the pathogens enter the body (Schaller, 2011).

To be effective, our behavioral defense needs to be able to detect perceptual cues that might imply the presence of pathogens. It also needs mechanisms that respond to the potential threat by helping the organism to engage in avoidance behaviors (Schaller & Park, 2011). Studies suggest that many animals, humans included, are able to gauge their conspecifics' health status based on perceptual cues (Altizer et al., 2003; Stevenson, Case, & Oaten, 2011). I will below describe in more detail how the detection and behavioral avoidance mechanisms work in animals and in humans.

3.1 ANIMALS AND A BEHAVIORAL DEFENSE AGAINST DISEASE

In animals, a behavioral prophylaxis against pathogens is present across species and is based on specific sensory modalities, namely olfaction and vision. Specifically, many species can use their olfactory system to detect disease cues. *Caenorhabditis elegans* relies on its olfactory system to detect pathogens, and interestingly, it avoids pathogen connoting odors (Bargmann, 2006; Zhang, Lu, & Bargmann, 2005). *Drosophila melanogaster* and *Scarabaeus (Kheper) lamarcki* can detect and avoid faeces containing phenol (which is associated with pathogens (Mansourian et al., 2016). Another interesting observation is that female mice can perceive olfactory sickness cues from male mice that carry parasites and they exhibit aversive behaviors towards them (Kavaliers & Colwell, 1995). Visual cues are also of importance for disease detection in animals. For instance, Gombe chimpanzees that were infected with poliolmyelitis acquired a peculiar way of locomotion or even lost the use of their limbs, were either avoided or being attacked by other chimpanzees, suggesting that their newly adopted pattern of movement served as a visual disease cue for the other chimpanzees (Goodall, 1986). On the whole, no matter how animals detect pathogens or what strategies they might use to avoid infection, the existence of a behavioral defense that includes detection and avoidance mechanisms is doubtlessly present among species.

3.2 HUMANS AND A BEHAVIORAL DEFENSE AGAINST DISEASE

In regards to disease detection and avoidance in humans, olfaction and vision play a major role (Schaller, 2011). Imagine for instance, opening the fridge to take the last steak you know is left. You might smell it and realize that the meat now has gone foul. You look at it and you realize that it is indeed putrid. You may immediately feel disgusted. Most probably you will decide not to cook and eat that steak because chances are that if you eat it you will get sick. In many cases, though, disease-connoting cues may be weak and less obvious. One example is when humans get sick and contagious with no obvious disease symptoms; an area which has just began to be explored with experimental scrutiny. In the three following sections, I will discuss disease detection and avoidance mechanisms that humans use in order to avoid contagion from other individuals. In particular, I will describe how olfactory and visual stimuli serve as sickness cues and how an avoidance behavior might be initiated.

3.2.1 THE SMELL OF DISEASE

Before I go more in depth about how olfactory sickness cues may be detected, it is necessary to first explain the nature of olfactory cues in this context.

Numerous Volatile Organic Compounds (VOC) are constantly emitted from our bodies and together form our individually unique body odor. These VOCs are emitted from various sources in our bodies such as breath, sweat, skin, and urine (Shirasu & Touhara, 2011). Considering that olfaction plays an important role in disease detection in some studied animal species, it could be speculated that smelling someone's breath or sweat can provide us with information regarding their health status. Indeed, bacterial and viral infections and other disease states – such as metabolic disorders or cancers – can affect the body odor (Shirasu & Touhara, 2011). Indeed, the body odor of people affected by typhoid fever has been described as smelling like freshly baked bread (Liddell, 1976; Pavlou & Turner, 2000). Smallpox has been described to give the skin a sweet and pungent odor (Tucker, 2002). Phenylketonuria, a metabolic disorder, has been described to give sweat a musty odor quality (Centerwall & Centerwall, 2000). People suffering from diabetes might have a breath that smells like a rotten apple or acetone (Buszewski, Kesy, Ligor, & Amann, 2007). To conclude, there is evidence that specific diseases are associated with specific and often negative odors that can be perceived by humans. One issue that arises from these observations, however, relates to the importance of the stage of the disease. In many cases the disease is already fully developed and the symptoms are obvious. However, the earlier we detect disease the more likely we are to escape contagion through avoidance.

Effective behavioral defense mechanisms should be sensitive to cues that even *imply* the presence of a pathogen (Schaller & Park, 2011; Thornhill & Fincher, 2014). Considering also that our olfactory systems might have evolved to act as a warning system (Hawkes & Doty, 2009), it is plausible to expect that even a subtle olfactory sickness cue should be adequate to activate avoidance. In fact, there exists evidence that attests to the latter. In that case, and before any studies will be presented, some explicit features of the odors, and specifically intensity and pleasantness, should be briefly addressed.

In studies using olfactory stimuli, scales of intensity are often used and olfactory disease detection studies are not an exception. The reason behind this is simply because by assessing the intensity in sick and in healthy body odor, information regarding a potential quantitative difference in health status can be retrieved (Olsson et al., 2014). Also in comparison with several animal species, studies support, perhaps surprisingly, that humans are capable of detecting odorous substances at extremely low concentrations (Devos, 1990; Laska, 2017). Intensity can also influence another aspect of olfactory perception: pleasantness. To present the same monomolecular substance at variable concentrations affects not only the intensity of the odor but also its quality, including pleasantness (Doty, 1975). Pleasantness is considered to be the foremost dimension of olfactory perception (Khan et al., 2007) and the level of pleasantness is the first characteristic individuals spontaneously attribute to an odor stimulus (Berglund, Berglund, Engen, & Ekman, 1973; Schiffman, Robinson, & Erickson, 1977).

An experimental study assessed whether humans are able to perceive olfactory disease cues within hours after the induction of an experimentally induced systemic inflammation using lipopolysaccharide (LPS) injections. Body odors were collected from the axilla region of volunteers. The body odors were then presented to naïve participants who rated the sick body odors as significantly more intense and less pleasant compared to body odors from healthy volunteers (Olsson et al., 2014). Building on that, a more recent study using a similar experimental setup investigated humans’ ability to detect differences in the urine volatile profile between experimentally sick (LPS) and healthy individuals. Results showed a difference in the natural daily rhythms of urine odor between sick and healthy condition, namely that urine from sick individuals was rated as more intense compared to urine from healthy individuals. This finding was paralleled by an altered urinary volatile profile of the sick individuals (Gordon et al., 2018).

Although more studies are needed, the results so far indicate that the human olfactory system might play a role in sickness detection with the potential to help humans stay healthy.

3.2.2 THE FACE OF DISEASE

It is evident that facial cues convey a great amount of information regarding, for example, emotional states (Ekman, 1992; Horstmann, 2003). But what about facial perception of health?

Studies have shown that specific facial cues, in particular coloration, adiposity and symmetry, can influence the perception of health of others (Henderson, Holzleitner, Talamas, & Perrett, 2016). Additionally, three studies that induced an experimental systemic inflammation by ways of an endotoxin (LPS) investigated whether humans' faces change in response to inflammation and whether this potential change is perceived by others. There is evidence that soon after a LPS injection, the human facial color turns more pale and less red suggesting that facial color could serve as sickness cue (Henderson et al., 2017). In another study, health ratings made by naïve participants were lower for the experimentally sick faces, indicating that humans can indeed perceive facial sickness cues just a couple of hours after the induction of systemic inflammation (Axelsson et al., 2018; Regenbogen et al., 2017). Finally, when the same faces were presented to a new set of naïve participants, that latter rated the sick individuals as having paler lips and skin, droopier corners of the mouth and more hanging eyelids, but also a more swollen face (Axelsson et al., 2018). Another study investigated whether humans can detect diseases that are not accompanied by a profound visible cue, such as sexually transmitted diseases. Results indicated that humans are indeed able to correctly identify as sick people with non-symptomatic herpes or HIV better than chance (Tskhay, Wilson, & Rule, 2016).

Sickness cues can also be retrieved from the perception of dynamic processes, such as walking or talking. Indeed, studies in which the health status of individuals was experimentally manipulated showed differential walking styles and walking speed (using objective measures) but also that the speed of walking of the sick individuals was rated as significantly slower and those individuals were perceived as more tired and less healthy compared to the same individuals when they were healthy (Lasselin et al., 2019; Sundelin et al., 2015).

In conclusion, detection of visual cues of sickness is likely an important part of the behavioral defense and its response to threats of a contagion.

3.2.3 DISEASE AVOIDANCE BEHAVIORS

Up till now I have discussed mechanisms that can be used to avoid contagion. It has been argued that the first step to avoid a pathogen is to detect it early. But what happens after that?

In the animal studies that were described above some defense strategies included physical avoidance of the sick conspecific but also aggressive behavior towards the sick animal. In humans, less is however known about avoidance behaviors.

Disease avoidance behaviors are believed to have a long evolutionary history (Curtis, de Barra, & Aunger, 2011). In response to disease detection, certain psychological processes take place. These include the arousal of the emotion of disgust but also the commencement of behavioral strategies that will guide the humans to avoid the risk of contagion (Schaller, 2011).

The emotion of disgust has been argued to be universal and a characteristic facial expression accompanies the experience of disgust (Ekman & Friesen, 1986). It has been suggested that disgust has evolved from the distaste response, since the emotion of disgust would prevent consumption of potentially dangerous food items (Rozin & Fallon, 1987). In relation to that, it appears to be a fundamental relationship between disgust and disease (Oaten, Stevenson, & Case, 2009). It has been suggested that disease connoting cues are also disgust elicitors (Curtis & Biran, 2001; Curtis, Aunger, & Rabie, 2004). Consider for example feces, a disgust-elicitor which is also a source of many bacterial and viral intestinal infections. Decayed meat, another disgust elicitor, might contain neuro- or entero-toxins and if consumed it can result in poisoning (Curtis & Biran, 2001).

In an online study where more than 40000 individuals took part, results showed that when photos of objects that carried an infection risk were presented to participants, they rated these photos as significantly more disgusting compared to photos with objects that were not associated with disease. The most interesting outcome of that study though was the universality of the response patterns across cultures (Curtis et al., 2004). Furthermore, dead bodies and biological decay are also considered disgust elicitors (Curtis & Biran, 2001). In relation to that, it has been suggested, but not shown, that the smell of death and decay can be a disgust elicitor (Haidt, McCauley, & Rozin, 1994). In sum, disgust is believed to have been evolved to motivate avoidance behavior that helps us stay healthy.

Liking has been suggested to predict approach and avoidance behaviors (Cialdini & Goldstein, 2004). A recent functional Magnetic Resonance Imaging (fMRI) study sought to investigate if sick humans are liked less by others compared to healthy humans (Regenbogen et al., 2017). Specifically, facial photographs and body odors of people with experimentally induced inflammation were simultaneously presented to naïve observers in the scanner. The naïve observers, immediately after the presentation of olfactory and visual stimuli, were asked to rate their liking towards the presented persons. As expected, sick faces were less liked and, moreover, sick body odors tended to further lower the liking of faces (Regenbogen et al., 2017). It is plausible that liking of someone is predictive of social approach behavior. With this background, the above study suggests that lower liking takes place when a disease threat is present, presumably as part of a disease avoidant mechanism.

A recent study from our group was conducted in an effort to study other aspects in line with decreased social contact of sick people. In particular, the same facial photographs as in the Regenbogen study were presented again to a different set of participants. This time the participants, using the same facial stimuli as in (Regenbogen et al., 2017) were asked to state how trustworthy sick and healthy persons were perceived. Results showed that sick people were rated as less trustworthy, and, thus, it could be speculated that they were also less likely to be approached (Tognetti et al., in prep).

Human evidence so far suggests avoidance behavior in response to disease threats in experimental studies. Individual differences and context effects may influence the decisions about the potential risk of contagion, as we shall see below.

3.3 OVERPERCEIVING CUES OF DISEASE

Different pathogens can cause different symptoms, humans can react in different ways to the same infection, and most importantly pathogens evolve rapidly (Ewald, 1996). Therefore, our behavioral defense may be constantly confronted with a broad span of uncommon appearances or overt behaviors that appear novel. In situations where humans may face ambiguity, a disease avoidance behavior can be employed even if there is no clear reason for it (Neuberg, Kenrick, & Schaller, 2011). Additionally, physical cues such as obesity, physical disability and aging (Duncan, Schaller, & Park, 2009; Duncan & Schaller, 2009; Park, Schaller, & Crandall, 2007) can be interpreted as disease cues. The emerging implication here is that in some cases cues that might connote the threat of disease but are not in reality contagious may trigger disease avoidance reactions. This example of liberal decision criteria in this context has been referred to as “overperception” of disease cues (Miller & Maner, 2012). Interestingly, overperception is usually the case when the perceivers of such cues perceive themselves as more vulnerable to disease (Schaller, 2011).

In fact, a basic principle that has been suggested to characterize our behavioral defense against infection is its functional flexibility (Schaller, Park, & Kenrick, 2007). Therefore, an up- or downregulation in innate responses can be observed when individuals perceive themselves as more vulnerable to disease. In addition, humans who perceive themselves to be, or who really are, vulnerable to disease may be more sensitive to disease connoting cues and consequently have a strong aversive response towards objects or people who might carry an infection threat (Schaller & Park, 2011). For instance, a study has shown that people who perceive themselves as more vulnerable to disease show anti-immigrant attitudes as they perceive foreigners as posing a disease threat (Faulkner, Schaller, Park, & Duncan, 2004). Similarly, another study showed that again people with higher perceived vulnerability to disease exhibit ethnocentric attitudes (Navarrete & Fessler, 2006; Thornhill & Fincher, 2014). The reason for that xenophobic attitude may be the belief that outgroup people might carry novel pathogens and therefore contact with foreigners might increase the exposure to this type of novel pathogens (Diamond, 1999).

Another trait that may interact with disease avoidance behavior and facilitate disease overperception biases, is disgust sensitivity. Humans that are high in disgust sensitivity may be more attentive towards objects or people who seem contagious and can be more sensitive to cues of sickness. Similar to perceived vulnerability to disease one might expect that people who are more concerned about their health would also exhibit higher disgust sensitivity.

We can conclude that similar to the immune system, the activation of the behavioral avoidance mechanisms comes at a cost, such as implications for social behavior and social activities. Some humans, of course, might commit false-positive errors instead of false-negatives since the benefit of staying healthy is greater than the cost of for example social withdrawal.

3.4 INTERACTION BETWEEN THE IMMUNE SYSTEM AND THE BEHAVIORAL DEFENSE AGAINST DISEASE

As previously discussed, when disease connoting cues are detected, certain psychological processes (including the emotion of disgust or aversive cognitions) are initiated in order to inhibit contact with the potential pathogen (Oaten et al., 2009). Although there is evidence for the psychological processes that take place when pathogens are detected, there are only a few studies examining the potential effect of disease detection on the immune system. In one study in particular, in two different conditions, photographs of either infectious disease symptoms or guns were presented to naïve participants. In both conditions neutral pictures, depicting furniture, were also presented. In vitro stimulated levels of pro-inflammatory cytokine IL-6 of participants were not elevated between pre- and post-measurements for neutral stimuli but were significantly more elevated in participants following exposure to pictures of disease symptoms compared to pictures of guns (Schaller, Miller, Gervais, Yager, & Chen, 2010).

In parallel, studies showed oral immune activation, in the form of elevated levels of salivary TNF-α and albumin, as a function of exposure to disgust images (Stevenson, Hodgson, Oaten, Barouei, & Case, 2011; Stevenson et al., 2012). Unpublished data from our group show similar results. Exposure to disgusting odors, compared to neutral, increased the levels of tumor necrosis factor alpha (TNF-α) in saliva (Juran et al., in prep). Another studyshowed elevated levels of salivary TNF-α, in response to disease and disgust related images, compared to neutral images, especially in participants with a higher tendency to feel disgust (Stevenson et al., 2015). Although more studies in that context are needed, there seems to be a linkage between disease detection and immune system activation. In other words, a behavioral defense does not only detect and avoid disease, but disease cues may in fact prepare the immune system for a pathogen attack.

4 SICKNESS BEHAVIOR

"I am a sick man.... I am a spiteful man. I am an unattractive man. I believe my liver is diseased. However, I know nothing at all about my disease, and do not know for certain what ails me."

Fyodor Dostoevsky, Notes from the Underground

No matter how well our immune systems works, we still get sick. Everyone who has been through a viral or bacterial infection would agree that getting sick is associated with specific changes in behavior. The most common changes that take place when people get sick are fatigue, nausea, malaise, anhedonia, lethargy, depressed mood, anxiety, disturbed sleeping patterns, and loss of appetite (Dantzer, 2009).

These behavioral changes that take place when we are sick, typically known as sickness behavior, are seen in both humans and animals and have evolved to enhance the work of the immune system against infections (Dantzer, 2004). Sickness behavior is triggered by pro inflammatory cytokines, such as IL-1, IL-6 and TNF-α (see Chapter 2.2). Through neural and humoral pathways, this immune activation is communicated to the brain, affecting neural targets resulting in modification of behavior (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). Most importantly, sickness behavior is considered as a motivational behavioral state that helps the organism to prioritize behaviors and actions that will promote recovery (Aubert, Goodall, Dantzer, & Gheusi, 1997; Aubert, Kelley, & Dantzer, 1997; Dantzer et al., 2008). It has also been suggested that sickness behavior is a form of behavioral defense not only of oneself in terms of recovery but also a defense for others, since social withdrawal protects the spread of contagion to kin (Shakhar & Shakhar, 2015).

4.1 SICKNESS BEHAVIOR IN ANIMALS

The most common way to study sickness behavior in animals is experimental sickness models where LPS injections are used to activate the immune system of the animal. Although all the behavioral changes in animals might not be as easy to investigate as in humans, and vice versa, consumption of food can be used, for example, to test for changes in motivation. In particular, loss of appetite is usually observed in rodents as indicted by lower food consumption (Kent, Bret-Dibat, Kelley, & Dantzer, 1996; Plata-Salamán, 1996). Propensity to fall asleep is also observed in animals in response to pro-inflammatory cytokines (Krueger & Majde, 2003). Finally, depression in animals is also suggested. For instance, the sick animals exhibit less social interactions and are more lethargic (Dantzer, 2001).

4.2 SICKNESS BEHAVIOR IN HUMANS

For humans, both clinical observations and experimental studies have shed light on sickness behavior. For example, it has been observed that almost one third of hepatitis C patients that are treated with immunotherapy experience depression (Asnis & De La Garza, 2006).

Experimental studies have also shown that mood is affected by immune activation. Analyses of two studies in which participants were injected with a LPS (0.4-0.8 ng/kg), an increase in state anxiety and a decrease in positive affect were observed (Lasselin et al., 2016). Other studies using a lower dose of LPS have also showed similar findings, with a lower increase in state anxiety compared to the aforementioned study (Karshikoff et al., 2015; Lasselin et al., 2016).

Other symptoms such as sleepiness or sleep patterns in general during sickness have also been investigated. It was shown that sleep disturbances in response to inflammation can be observed (Mullington et al., 2000; Pollmächer et al., 2000). The disturbed sleep has recently been investigated in individuals who experience naturally occurring respiratory infections. Indeed, a worse sleep quality was reported when individuals were sick compared with when they had recovered from the infection (Lasselin et al., 2019).

Another study that tested the motivation behavior in a monetary task in young adults that were injected with LPS found that sick individuals were engaged to high effort tasks when the reward was also high (Lasselin et al., 2017). Similarly to animals, appetite is also decreased in humans in response to inflammation (Reichenberg et al., 2002). Finally, preliminary results show that, following LPS administration, individuals showed an increase in anhedonia and fatigue and a decrease in social interest (DellaGioia, Devine, Pittman, & Hannestad, 2013).

A note of caution should be made at this point though. Sickness behavior could be maladaptive if left unabated. Thus, it may play a role in major depression where about in 1/3 of patients exhibit low-grade inflammation (Osimo, Baxter, Lewis, Jones, & Khandaker, 2019). In conclusion, there is evidence that sickness behavior is present across species and that a communication between the immune system and the brain has evolved which helps us develop responses that promote and facilitate recovery (Dantzer & Kelley, 2007). Pertinent to this thesis is that chemosensory perception has not been investigated in the context of sickness behavior.

5 AIMS

5.1 OVERALL AIM

The overall aim of this thesis was to investigate how humans are able to discriminate between sick and healthy individuals based on sensory cues and whether the detection and effects of these cues are in line with the notion of a behavioral defense against disease. The sensory cues studied were either visual (Study I) or olfactory (Study III), or both visual and olfactory cues (Study II). Another main aim was to investigate chemosensory sickness behavior, that is, whether sickness affects the perception of odors and tastes (Study IV).

5.2 SPECIFIC AIMS

Study I

In Study I, the aim was to investigate whether facial emotional expressions during experimentally induced inflammation were different compared to the expressions of healthy individuals, as judged by naïve observers. Moreover, a possible mediation of emotions to sickness perception was also investigated.

Study II

In Study II, a combination of both visual and olfactory disease cues were used to assess the effects of sensory cues on social liking. As a secondary aim, individual traits such as perceived vulnerability to disease, disgust sensitivity, and health anxiety were also assessed in relation to the liking ratings in order to investigate if the traits can affect the liking of others.

Study III

In Study III, olfactory detection of a naturally occurring infection was tested. The aim here was to investigate body odor as a possible sickness cue in a respiratory infection. As in study II, the effects of certain individual traits (perceived vulnerability to disease, disgust sensitivity, and health anxiety) on the olfactory disease perception were also assessed.

Study IV

In Study IV, the aim was to investigate how experimentally induced inflammation can influence odor and taste perception in humans in line with previous observations concerning loss of appetite and social withdrawal as part of sickness behavior.

6 METHODS, RESULTS, AND CONCLUSIONS

6.1 STUDY I: EMOTIONAL EXPRESSIONS OF THE SICK FACE

6.1.1 Methods

Twenty–two healthy volunteers (9 women, mean age 23 years) took part in the sampling phase. All volunteers had to be between 18 and 50 years old, non-smokers, non-excessive alcohol users, and non-obese. The volunteers were tested during two separate study days at the Centre for Clinical Research, Danderyd Hospital, Stockholm, Sweden. They were randomly assigned to either receive a lipopolysaccharide injection (*Escherichia coli* endotoxin, Lot HOK354, CAT number 1235503, United States Pharmacopeia, Rockville, MD, USA) at 2.0 ng/kg body weight or a placebo injection (0.9% NaCl) on the first day. A month later, the second session took place where volunteers received the reverse treatment. In both conditions (LPS and Placebo), pro inflammatory cytokines [interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), interleukin-8 (IL-8)], subjective sickness ratings, and tympanic temperature were measured.

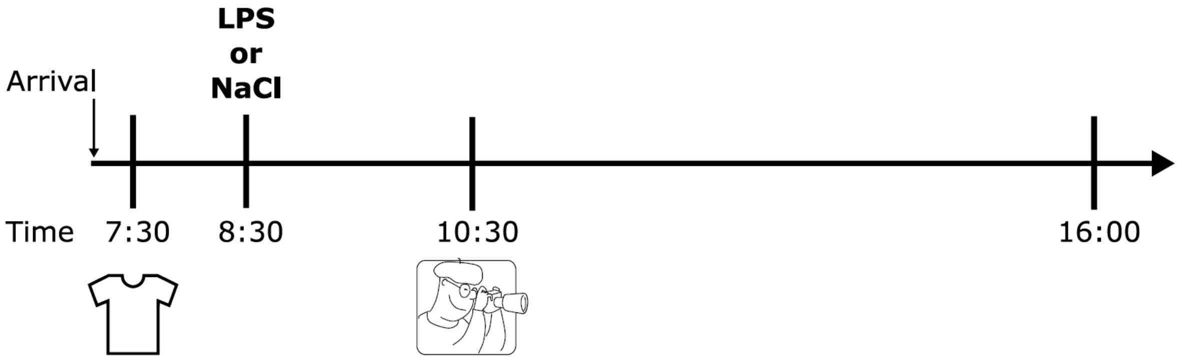

Figure 1: Overview of the first part of the study. T-shirts with cotton pads in the armpit region were given to the volunteers upon arrival to the hospital. An hour later the treatment (LPS or saline injection) took place, and two hours later the facial photographs of the volunteers were taken.

Facial photos of the volunteers were taken about two hours after injection at the approximate peak of the pro-inflammatory cytokine levels (Figure 1). Photos were taken while the volunteers were asked to sit comfortably, look straight into the camera with a neutral facial expression. They wore identical white t-shirts and no make-up. Four volunteers were excluded due to subtle differences in lighting of the photos, and differences in facial hair between the two conditions. In total, 36 photos were used in study I (from 18 individuals participating in both LPS and the Placebo condition). Photos were cropped to only include the face (see examples in Figure 2), ensuring thus that the raters in the next part of the study would not be influenced by non-facial cues.

Figure 2: Healthy (left) and sick (right) face as a result of experimental induction of systemic inflammation.

Forty-nine individuals (31 women, mean age 31.2 years) participated in study I as raters. The raters were tested in groups in 8 different experimental sessions. Every rater participated only in one session. The group size varied from 3 to 14 individuals and the raters were not seated directly next to each other. In each session the raters viewed a screen presentation of the photos that served as stimulus material, one face at the time. Each photo was shown for 45 seconds during which the ratings were performed. Between each presentation a fixation cross was shown for 3 seconds. The order of the 36 photos was randomized for each session. Each rater filled out a questionnaire on paper consisting of 36 parts (one for each photo). For each part, one face was rated with regards to what extent different emotional expression were evident: happy, angry, sad, scared, disgusted, surprised, and neutral, using a Visual Analogue Scale (VAS) ranging from 0 ("not at all") to 10 ("to a high extent").

The raters also rated level of sickness/health for each photo on a VAS ranging from -5 to 5, where -5 meant "very sick", 5 meant "very healthy", and 0 meant "neither sick nor healthy" (see example in Figure 3).

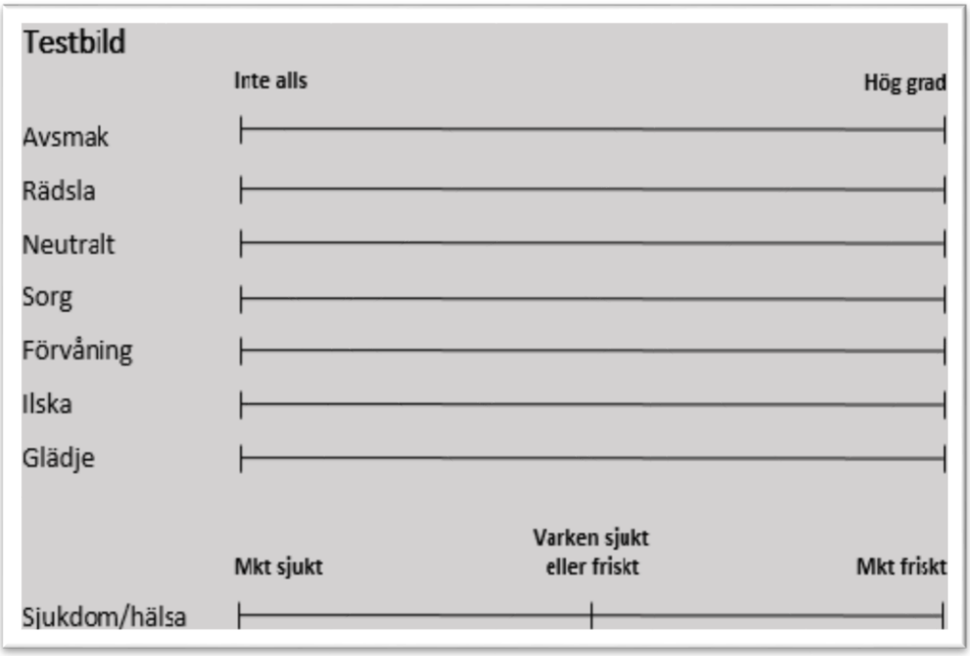

Figure 3: The visual analogue scales used in the study (in Swedish).

6.1.2. Results

The faces in the LPS condition were perceived as expressing more negative and less positive emotions. They were rated as significantly sadder (d = 0.151, p < .001) and more disgusted (d = 0.148, p < .001), and as significantly less happy (d = -0.365, p < .001) and surprised (d = -0.142, p < .001) compared to the faces in the placebo condition. The faces in the LPS condition were also rated as significantly more sick compared to the faces in the placebo condition (Cohen's d = -.261 p < .001).

Moreover, more than half of the total effect of treatment-dependent variation was mediated through the rated emotions (59.1 %). Ratings of more sadness and disgust, and of less happiness and surprise significantly mediated the effect of LPS on increased sickness perception – while approximately 40 % of the effect was direct.

6.1.3. Conclusions

The main conclusion of Study I was that systemic inflammation leads to a change in the facial emotional expression. Sick faces look sadder, more disgusted and less happy which potentially inhibits social contact thereby decreasing risk of contagion. Additionally, one can draw the conclusion that humans might, indeed, be capable of detecting visual disease cues and facial emotional expressions might play a crucial role in disease detection.

6.2 STUDY II: PEOPLE EXPRESSING OLFACTORY AND VISUAL CUES OF DISEASE ARE LESS LIKED

6.2.1 Methods

Seventy-seven healthy participants took part in the present study (mean age 30 years; of which, 49 women). Inclusion criteria were ≥18 years of age, self-reported normal or corrected to normal vision, self-reported normal sense of smell, non-smokers, not pregnant, and finally, an ability to speak and understand Swedish.

The study consisted of two sessions. During the first session, participants completed several questionnaires collecting socio-demographic data and measuring their sensitivity to disgust (DS-R), health anxiety (HAI) and perceived vulnerability to disease (PVD). After the first session was completed, the experimenter prepared the participants for the recording of facial electromyography (EMG). During the second session, they gave liking ratings following the simultaneous presentation of face and body odor stimuli while EMG was recorded.

The face stimuli were the same as the ones presented in Study I. For the sampling of the olfactory stimuli, all donors wore tight T-shirts (type BB301, 50% cotton/50% polyester; American Apparel, London, UK) for five hours, with nursing pads sewn into the armpit regions, following treatment both for LPS and placebo. After the completion of sampling, all pads were removed from the T-shirts and were stored in a freezer at -35 ºC in 1-L freezer bags.

6.2.2 Results

As hypothesized, there was a main and negative effect of sickness on liking of face sickness ($t(22.4) = -3.586$, $p = .002$, $d = -0.40$), as well as for body odor sickness ($t(22.9) = -2.202$, $p = .038$, $d = -0.25$) compared to the healthy ones. There was no significant interaction effect between face and odor sickness on liking ratings ($t(70.3) = 1.204$, $p = .232$, $d = 0.15$). In sum, the results indicate that both face and odor sickness decrease liking of persons. Moreover, four linear multiple regression models were performed, one for each of the questionnaire's subscales and one for the three total scores of each questionnaire. Mean liking ratings were the dependent variable in all models. In the regression analysis, the PVD germ subscale was significantly and negatively associated with overall liking of the presented persons.

6.2.3 Conclusions

In Study II it was shown that both olfactory and visual cues are present after an experimentally induced inflammation. As a result, sick people were less liked. This is true for both visual and olfactory sickness cues. However, no interaction between these types of sickness cues was found. Moreover, people who perceive themselves as more vulnerable to disease tended to dislike others in general, possibly suggesting that these individuals are less likely to socialize.

6.3 STUDY III: OLFACTORY COMMUNICATION OF SICKNESS CUES IN RESPIRATORY INFECTION.

6.3.1 Methods

The stimuli for the current behavioral experiment were obtained from a study of respiratory infection (Lasselin et al., 2019). Twenty-three volunteers donated body odors at two different sessions (sick and healthy) separated by four weeks. To be eligible for donation of body odors, all volunteers had to have at least one of the following respiratory symptoms: cough, sore throat, shortness of breath, or coryza. They also had to experience one of the following systemic symptoms: fever, headache, malaise or myalgia. All volunteers were asked to avoid taking antipyretics and nasal sprays.

Fifty-four individuals were recruited as participants for the present study. Due to problems with the software tool used, only forty-six participants provided responses eligible for statistical analysis. To be included in the study participants had to be non-smokers, not pregnant, have a normal or corrected to normal vision, a normal sense of smell and be 18 years old or older.

All participants were presented with the odor stimuli (23 sick and 23 healthy, described above) in a unique randomized order. The odors were presented for 3 seconds each. After the presentation of the body odors the participants were presented with four visual analogue scales at a time, where they had to rate the intensity, pleasantness, health, and disgust of the body odors using a computer mouse (software used: E-prime Psychology Software tools, Sharpsburg, PA, USA). All participants had four seconds to give ratings for each scale. The ratings were ranging from 0 to 100, were 0 would indicate not intense, not pleasant, not healthy and not disgusting at all, 100 would indicate the opposite, very intense, very pleasant, very healthy, and very disgusting, and 50 would indicate a neutral experience. All participants filled in the same questionnaires (PVD, DS-R, HAI) as the participants in Study II.

6.3.2 Results

In line with the hypothesis, body odors of sick volunteers were nominally more intense ($\chi2(1) =1.99$, $p = 0.16$) and disgusting ($\chi2(1) =3.62$, $p = 0.06$), as well as less pleasant ($\chi2(1) = 0.41$, $p = 0.52$) and healthy ($\chi2(1) =2.14$, $p = 0.14$) but the results in all cases were statistically insignificant. The analyses regarding the influence of each of the three different questionnaires, revealed no significant effect of them on the four different rating scales.

6.3.3 Conclusions

Although the results of the present study did not reach significance, it could be noted that the nominal effects were in the hypothesized direction. One possibility for that the negative change of body odor found in previous LPS studies (e.g., Study II) is not observed here as a function of respiratory infection is that that the level of inflammation in this study was low. More studies are obviously needed to map out the relation between level of inflammation and strength of disease cues.

6.4 STUDY IV: SICKNESS BEHAVIOR AND CHEMOSENSORY PERCEPTION

6.4.1 Methods

Forty participants were recruited for the present study. For inclusion, participants had to be between 18-35 years old and healthy. Twenty-five participants were normal weight, and fifteen were obese (BMI > 30 kg/m^2). The stimuli material for Study IV were obtained from another study in which the effects of obesity on sensitivity to sickness behavior were assessed. Exclusion criteria were smoking and excessive alcohol consumption, pregnancy, physiological or psychological disease, current medication or abnormal blood analysis. One participant failed to deliver complete ratings and was therefore excluded from the analysis.

Using a double-blind randomized, placebo controlled, crossover design, all participants were injected intravenously with either lipopolysaccharide (LPS, Endotoxin Reference Standard, *Escherichia coli*, CAT number: 1235503, lot H0K354, United States Pharmacopeia, Rockville, MD, USA) or saline (placebo). The dose of LPS injection was 0.8 ng/kg body weight for the normal-weight participants. The LPS dose for the obese participants was adjusted for estimated blood volume and it varied from 0.51 to 0.68 ng/kg body weight. The reason for that is that obesity is associated with smaller proportion of blood volume relative to weight. All participants received both treatments in a randomized order with 1-2 weeks of wash-out.

The stimuli were comprised of 8 odors in jars and 4 aqueous taste solutions and a control solution in spray bottles. The 8 odors (chocolate, PEA, pyridine, sour cream, sweat, urine, cotton, blood) were stored in a fridge and were taken out to reach room temperature before testing. The 4 taste solutions were basic tastes; bitter, sour, salty, and sweet. The control solution was water.

The odors were presented in a random order for each participant. After the experimenter had placed the odor jars 2 cm below their nostrils, he/she asked the participants to sniff. The participants were told that they could sniff two times if needed. After the presentation of each odor stimulus the participants had to indicate how intense or how pleasant the odor was on a scale from 1 to 10. The taste stimuli were also presented in a randomized order. The participants were asked to close their eyes and the experimenter sprayed onto the participants' protruded tongue. The spray bottle was hold approximately 5-8 cm away from the tongue. After the presentation of each taste stimulus, the participants were asked to rinse their mouth with water before they proceeded to the next stimulus. Intensity and pleasantness were rated for each taste stimulus. There was a 30 seconds time interval between each stimulus presentation.

6.4.2 Results

Linear mixed model analysis for intensity and pleasantness showed a significant and negative effect of sickness on the perception of pleasantness on food odors (chocolate, sour cream). Analyses of the effect of sickness on taste perception did not show any significant results.

6.4. Conclusions

In Study IV, the main conclusion is that as a function of sickness, intensity and pleasantness ratings of odors and tastes were largely unaffected. Food odors were overall perceived as less pleasant in line with the observations of lower appetite as a part of sickness behavior.

7 DISCUSSION

The overall aim of this thesis was to investigate if humans can discriminate between sick and healthy human conspecifics based on sensory sickness cues. We further aimed to test if sickness-related traits related to disgust sensitivity, perceived vulnerability to disease and health anxiety can influence how sickness cues are perceived. We finally investigated if perception of olfactory and gustatory stimuli is modified during sickness. The results showed that humans indeed can dissociate between sick and healthy individuals and that facial perception and olfactory perception are two different modalities for picking up sickness cues just hours after induction of systemic inflammation. Interestingly, the results also suggest that sick individuals are perceived as less likable and as expressing less positive emotions. These observations predict more of social avoidance and less of approach when cues of infection are encountered, which in turn would limit contagion.

Before I start discussing in more detail the findings of the studies, there is one important clarification that needs to be made and that is the distinction between cues and signals. In the framework of disease detection one should be cautious with the use of these two terms. In animals, humans included, communication, and as a result interaction with others, is based either on signals or cues (Schaefer & Braun, 2009). Signal is everything that has evolved to play a very particular role in communication, for example pheromones (Karlson & Luscher, 1959). Therefore, a signal *intentionally* changes or upholds a certain behavior of the perceiver (Abrantes, 2011). Cues, on the other hand, have not evolved for reasons that relate to signalling (Schaefer & Braun, 2009) and their presence does not necessarily carry a specific meaning (Saleh, Scott, Bryning, & Chittka, 2007). Consequently, it is upon the perceiver to interpret cues. So, a cue *unintentionally* changes or upholds a certain behavior of the perceiver (Abrantes, 2011). It is possible that some disease cues have evolved to alert kin to beware of contagion or caretakers to be alerted of ill-health in their offspring. But, as this is unclear in the current studies and because "cue" is a lesser claim, I have used the word cue.

Study I focussed on facial cues as a function of induced sickness. The results on sickness-induced emotional expressions (more sadness and disgust and less happiness and surprise) add to the current state of our knowledge regarding disease detection in humans. In parallel (as discussed in Chapter 3), previous studies of induced inflammation have shown that visual cues such as facial features, facial coloration and gait patterns can also be indicative of sickness (Axelsson et al., 2018; Henderson et al., 2017; Lasselin et al., 2019; Sundelin et al., 2015). Indeed, our results showed that perceiving someone as sick or healthy was mediated by mostly negative facial expression. It could be argued, though, that this result was due to a halo effect, suggesting that an individual with a face that was perceived as sick, for instance by way of paleness, was thought to (rather than perceived to) host negative emotions. However, mediation analyses accounting both for changes in the facial physical features and perceived emotions showed that physical changes are a part of the mediation between sickness and perceived health. As such the sickness-related emotional expressions of others may increase the probability of avoidance of sick persons. Altogether these studies have started to map out the visual expressions of sickness that can be seen just a few hours after the induction of sickness.

As noted, the sick faces were also rated as expressing significantly more of disgust compared to the healthy faces. The emotion of disgust is believed to be a central part of the behavioral defense against disease. Although one could argue that the disgust expression is derived from simply being sick and feeling nauseous (Widen, Pochedly, Pieloch, & Russell, 2013), and as such it should be considered a cue, it cannot be excluded that sick individuals express more disgust as part of a sickness behavior in an effort to signal the ill-health status to kin (Shakhar & Shakhar, 2015).

Taking into consideration studies showing that the perceiver of an emotional face also exhibits a subtle but measurable version of that emotion (Dimberg, Thunberg, & Elmehed, 2000; Moody, McIntosh, Mann, & Weisser, 2007), it would be interesting, to test if the perceiver also feels disgusted by looking at a sick face expressing disgust in a future study.

In Study II, we investigated whether visual (faces) and olfactory (body odor) sickness cue types can add to each other and result in less liking of the sick person and presumably disease avoidance. Our results showed that sickness cues both in face and in body odor had a negative effect on the liking of the sick person. This is in accordance with previous studies suggesting that olfactory cues can help us detect disease (Gordon et al., 2018; Olsson et al., 2014) but also with studies suggesting that the face express the health status (Axelsson et al., 2018; Regenbogen et al., 2017; Sarolidou et al., 2019).

Perceived vulnerability to disease, can influence the way humans respond to a real or potential threat of disease. In particular, differences in perceived vulnerability to disease can predict preferences towards facial features that connote health (Duncan et al., 2009). Based on these observations, in Study II we also sought to examine if certain personality traits, such as Perceived Vulnerability to Disease, Disgust Sensitivity, and Health Anxiety, could influence the liking of others. We found a negative relationship between individuals' perceived vulnerability to disease and liking ratings. This could be interpreted as if our own experience of disease vulnerability might bias us to avoid others. The latter is in accordance with previous research suggesting that humans who score high on the PVD questionnaire tend to avoid both conspecifics and other things that might carry an infection risk (Schaller, Murray, & Bangerter, 2015). It should be pointed out, though, that this negative relationship was observed only in one out of the two studies included in this thesis, and although it is an interesting finding, it should be interpreted with caution.

In the first two studies, we used as visual stimuli facial photographs of volunteers who were made experimentally sick by LPS injections. One limitation of these specific stimuli is the restricted age range of the presented faces. All of them depicted young adults, and that leaves a question regarding how good we are at detecting cues of sickness in older adults whose faces may, for example, have droopier mouth and, consequently, may be mistaken for emotional expressions (Malatesta, Fiore, & Messina, 1987). Apart from being more or less the same age, the volunteers were all Caucasian. Considering that, as discussed in Chapter 3, facial coloration and other specific facial features are cues of sickness (Axelsson et al., 2018; Henderson et al., 2017), it would be interesting for future studies to investigate the facial cues of disease in people of different color and ethnicity.

In Study III, we investigated if it is possible to detect disease in individuals having a naturally induced inflammatory response, i.e., a respiratory infection. Similar to Study II, we first tested whether participants could perceive olfactory disease cues, but also whether individual traits could influence disease detection. Intensity, pleasantness, health, and disgust scales were used to test for perceptual differences between sick and healthy body odors. Although the results were nominally in the hypothesized direction (sick body odors were rated as more intense, less pleasant, less healthy, and more disgusting), the effect was not statistically significant.

The absence of a sickness-related effect on body odor in respiratory infection could either be generic to respiratory infection or be related to the level of sickness. Looking at the degree of inflammation in the experimental disease studies of the present thesis, and the probable absence of a high degree of inflammation in Study III, one can see that the sickness symptoms were significantly lower in the upper respiratory infection. Perhaps, in order to be able to detect disease in others, a stronger disease state, and a stronger accompanying inflammatory response, is necessary. The people who experienced respiratory infection did not develop much fever and their subjective sickness symptoms were minor compared to the individuals who had an experimentally induced inflammation.

To be able to develop the knowledge regarding the role of inflammation in how disease states can be detected by others, future studies should try to specifically address the level of inflammation in regards with disease detection. Since in Study III measurement of inflammatory markers was not possible, valuable information regarding the degree of inflammation were missing. An experimental study in which both a low and a high dose of endotoxin are administered to different sets of volunteers and potential differences in disease detection due to degree of inflammation are assessed, may shed light on this problem. In addition, future studies should also address disease detection in a naturally occurring inflammation including higher levels of inflammation.

In Studies I-III the focus was on disease detection and its relation to disease avoidance. The main conclusion from these studies is that humans might be able to detect sickness in others and as a consequence they might be able to avoid their sick conspecifics. One might wonder though if avoidance behavior is the only type of behavior that it is shown towards sick peers. Animal studies have shown that helping a sick kin might also be an adaptive strategy against pathogens (Hart, 1990). There is evidence from rodent studies which indicate that mothers of sick (LPS-treated) rat pups exhibit increased care behavior in that they lick their offspring significantly more compared to the mothers of healthy rat pups (Breivik et al., 2002). This reaction to disease is also in line with informal observations from many of us. This indicates that disease detection might also lead to approach behavior. It is plausible, therefore, to claim that our behavioral defense against disease could be a motivational system that helps us detect disease and act adaptively, either by avoiding the risk of contagion or by helping the sick person to recover.

As discussed in Chapter 3, our behavioral defense against disease employs different sensory cues to detect disease. It is generally known that when weak cues from different sensory modalities are combined, faster and more accurate detection can be achieved (Stein & Stanford, 2008; Stevenson et al., 2014). Based on that idea, the multisensory integration in the context of disease detection was assessed in the present thesis (Study II) in order to test if disease detection is particularly indicated when visual and olfactory cues are combined. When both visual and olfactory sickness cues were simultaneously presented to naïve participants, some degree of additivity was observed, but no significant interaction that would have suggested a synergy between these cues. Considering that the sick faces and the odors that served as stimuli in Study II were both less liked and perceived as more sick than the healthy versions we would have believed that these bimodal cues could add more potently to each other. A possible explanation for that relative lack of synergy is that the mechanisms that led to less liking of the sick faces and body odors may have been *different* for each sensory modality. For instance, it has been shown that sick body odors are perceived as more “unpleasant”, a primary dimension in odor quality (Gordon et al., 2018; Olsson et al., 2014), and sick faces as more “sad and disgusted” (Sarolidou et al., 2019). One could speculate that these different “channels” do not allow for higher levels of additivity. However, more studies are needed in order to investigate how sensory cues are integrated and how multisensory integration can enhance disease detection.

When trying to generalize the experimental sickness model to a natural sickness model, the mapping on the time scale of the progression of the natural sickness should be considered carefully. In the LPS model used in the present thesis, the peak of the inflammatory response according to the measures of the pro-inflammatory cytokines was about two hours after the induction of inflammation (when facial photographs were taken). The effect of LPS then washes out during the following hours. Natural sickness typically has a longer progression and is peaking later. When we think of cues sampled two hours after the experimental induction of inflammations as “early” it is because the immune system has launched its response just recently. But is difficult to determine how to map this particular state of inflammation to the course of any particular natural sickness. From this perspective, LPS models needs further validation against natural sickness.

In Study IV the focus was shifted from disease detection and avoidance to the related area of sickness behavior. More exactly, we investigated for the first time how the sick person perceives chemosensory stimuli. Considering that pro-inflammatory cytokines inhibit the rewarding mechanisms of food intake (Merali, Brennan, Brau, & Anisman, 2003) it is plausible that appetite is decreased when we get sick. In addition to inhibition of rewarding mechanisms of food intake, pro-inflammatory cytokines also mediate anorexia during inflammation (Wong & Pinkney, 2004). Specifically, TNF-α can produce an anorexigenic effect (Romanatto et al., 2007) and consequently elevated levels of TNF-α are observed in anorexia patients (Khalil, de Muylder, & Hebborn, 2011). It could therefore be argued that food-related chemosensory stimuli, in particular, would be rated as less pleasant.
With this background, odors (food and social odors) as well as tastes (sweet, sour, bitter, and salty) were presented to volunteers when they were experimentally sick and when they were healthy. From the previous studies just noted, and from common belief, we would have expected clear effects on the appreciation of stimuli reminding of foods, such as food odors, sweet, sour, salty, and bitter tastes. However, a significant but weak effect was observed only for food odors, indicating that sick individuals on average perceive food

odors as less pleasant. One explanation for the lack of stronger findings could be that when sick, the sniffing of odors was reduced, and in consequence, that could have counteracted a potential effect on the pleasantness of odors. Indeed, unpleasantness of odors is correlated with a reduced (shorter and shallower) sniffing (Prescott, Burns, & Frank, 2010). Another point that should be discussed in relation to these findings is the difference between wanting and liking. Research has shown that different neural circuits are involved when something is wanted compared with when something is liked (Berridge & Robinson, 2016). For instance, dopamine suppression both in Parkinson's disease patients and in healthy participants showed no decrease in pleasantness ratings of delicious foods (Hardman, Herbert, Brunstrom, Munafò, & Rogers, 2012). If pleasantness ratings tap more into liking than wanting, it could be hypothesized that pleasantness ratings of food-related chemosensory stimuli such as those used in Study IV may not change much when we get sick.

In Chapter 4, sickness behavior was described as a motivational system that promotes recovery, i.a., through social withdrawal. As noted, it has also been suggested that sickness behavior does not only promote recovery, but may also be an adaptation that eliminates the spread of contagion to kin (Shakhar & Shakhar, 2015). Both these motivational aspects of sickness behavior promote social withdrawal. With this background, it could have been expected that sickness would have a negative effect on the perception of social odors (sweat, urine, and blood) that were presented to the sick individuals. No such effects were found in Study IV, however. Admittedly, all odors classified as social were synthetic odors and it is unclear which odors should be really be regarded as social among humans.

During the last decade interest in the workings of a behavioral defense against disease has increased. As mentioned in Chapter 1, the set of behaviors that humans and animals have at hand is often referred to as Behavioral Immune System (Schaller, 2006). What shall be counted as part of this defense or system, and why, is not clear. Sickness behavior, i.e., behavior once infected, could fit in to the concept of a behavioral defense, but is typically not. Another aspect on a behavioral defense concerns whether the mechanisms are multi-purpose or dedicated. For instance, detecting facial sickness cues such as pale lips and droopy corners of the mouth is most likely performed by multi-purpose perceptual mechanisms evolved to discriminate between colors or levels of lightness of any two surfaces. Other mechanisms of the defense could be argued to be more dedicated, such as feelings of disgust in response to disease-relevant stimuli and also immunological reactions to sickness and disgust stimuli. Future works in this field should better define the theoretical framework of a behavioral defense against disease.

The identification of mechanisms of a behavioral defense could also have some clinical importance. Specifically, the relationship between health anxiety and disease avoidance mechanisms could be of interest. People suffering from health anxiety are under a constant fear of somatic illness. A study in which participants suffered from severe health anxiety, showed that disgust might play a role in this disorder (Hedman et al., 2016). So, what if a maladaptive response to disease related stimuli is to some degree responsible for health anxiety? Studies as those described in the present thesis could be instrumental when investigating the mechanisms of psychiatric disorders such as health anxiety and perhaps result in the formation of a better treatment.

In conclusion, behavior, which includes not only the behavioral avoidance of infectious things, but also the sickness behavior, could be argued to be a part of a defense against disease. As such it is a truly first line of defense with clear benefits but also some costs, such as implications for social life. Sickness can be detected in multiple ways by several sensory systems thereby promoting detection sensitivity. Interestingly, cues are available already a few hours after the induction of systemic inflammation, without the cumulative effects of prolonged immune activation that could possibly be part of a manifest disease and its related cues (such as cough and runny nose). Perhaps, the early detection seen after experimental immune activation might speak for a mechanism that facilitates detection of pathogens in peers during the most contagious state. However, the timeline of how cues appear in natural sickness needs to be further investigated. The nature of the cues observed here promotes dislike of sick individuals which suggest that the cues likely feed into a motivational system that in general promotes either increased avoidance behavior or decreased approach behavior. As such these cues may be pivotal in a behavioral defense against disease that helps us stay alive and healthy.

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